

Claims

- Sub B1
1. A process for the isolation of nucleic acids from a sample including the following steps:
- (a) applying at least one nucleic acid sample to a membrane;
 - (b) immobilizing the nucleic acids on the membrane;
 - (c) releasing the immobilized nucleic acids from the membrane; and
 - (d) removing the released nucleic acids through the membrane, whereby the membrane is comprised of one or more materials selected from the group consisting of nylon, polysulfone, polyethersulfone, polycarbonate, polyacrylate, acrylic copolymer, polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate, poly-tetrafluoro-ethylene, polyvinylidene fluoride, polyethylene-tetrafluoro-ethylene-copolymerisate, polybenzimidazole, polyethylene-chlorotrifluoro-ethylene-copolymerisate, polyimide, polyphenylene sulfide, cellulose, cellulose-mix ester, cellulose nitrate, cellulose acetate, polyacrylnitrile, polyacrylnitril-copolymer, nitrocellulose, polypropylene and polyester.
2. The process according to Claim 1, characterized in that the nucleic acid sample is applied on top of the membrane and the nucleic acids are removed from below the membrane.
3. The process according to Claims 1 or 2, characterized in that the membrane is placed in a container with an inlet and outlet and the membrane fills the entire cross-section of the container, separating said inlet and outlet.
4. The process according to Claim 1, characterized in that the membrane is coated.
5. The process according to Claim 4, characterized in that the membrane has been made hydrophobic by the coating.
6. The process according to Claim 4, characterized in that the membrane has been made hydrophilic by the coating.

7. The process according to Claim 1, characterized in that the membrane is less than 1 mm thick.

8. The process according to Claim 7, characterized in that the membrane is less than 0.5 mm.

Sub B2
9. A process for the isolation of nucleic acids from a sample comprising the following steps:
(a) applying at least one nucleic acid sample to a surface;
(b) immobilizing the nucleic acids on the surface;
(c) releasing the immobilized nucleic acids from the surface with an elution agent, characterized in that the release takes place at a temperature T , whereby $10^{\circ}\text{C} \geq T \geq T_{\text{s,EM}}$, and $T_{\text{s,EM}}$ equals the freezing point of the elution agent.

10. The process according to Claim 9, characterized in that the release takes place at temperature T , in which $10^{\circ}\text{C} \geq T \geq 5^{\circ}\text{C}$.

Sub C2
11. The process according to Claims 9, characterized in that the release takes place at temperature T , in which $10^{\circ}\text{C} \geq T \geq 0^{\circ}\text{C}$.

12. The process according to Claims 9, characterized in that the release takes place at temperature T , in which $10^{\circ}\text{C} \geq T \geq -5^{\circ}\text{C}$.

13. The process according to Claim 9, characterized in that the release takes place at temperature T , in which $5^{\circ}\text{C} \geq T \geq T_{\text{s,EM}}$.

Sub B3
14. A process for the isolation of nucleic acids from a sample comprising the following steps:
(a) adjusting a nucleic acid sample to binding conditions that permit immobilization of the nucleic acids contained in the sample on a surface;
(b) applying the nucleic acids sample to the surface; and
(c) immobilizing the nucleic acids on the surface,

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characterized in that, before and/or after adjusting the binding conditions there is a pre-treatment of the sample.

15. The process according to Claim 14, characterized in that the pre-treatment takes place by salting out.

16. The process according to Claim 14, characterized in that the pre-treatment takes place by way of filtration, centrifugation, enzymatic treatment, temperature effect, precipitation, extraction, homogenization, mechanical reduction and/or binding of contaminants to surfaces.

17. The process according to Claim 14, characterized in that the binding conditions permit immobilization of RNA.

18. The process according to Claim 14, characterized in that the binding conditions permit immobilization of DNA.

19. The process according to Claim 14, characterized in that the following additional steps are included:

- releasing of immobilized nucleic acids from the surface, and
- removing the released nucleic acids from the surface.

sub C3
20. The process according to any one of Claims 1, 9 or 14, characterized in that after the release step at least one additional step takes place:

- performing at least one chemical reaction with the nucleic acids.

21. A process for performing a nucleic acid amplification reaction comprising following steps:

- (a) applying at least one nucleic acid sample to a surface;
- (b) immobilizing the nucleic acids on the surface; and
- (c) performing an amplification reaction with the nucleic acids.

22. The process according to Claim 21, characterized in that the amplification reaction is not isothermal.
23. The process according to Claim 21, characterized in that the amplification reaction is isothermal.
24. The process according to Claim 21, wherein the nucleic acid amplification reaction is a Strand Displacement Amplification (SDA) reaction, a PCR, or an RT-PCR.
25. The process according to Claim 21, characterized in that, prior to performing the amplification reaction, the nucleic acids are released with a suitable reaction buffer from the surface and the eluate is located on or in the membrane.
26. The process according to Claim 25, characterized in that there is an additional step of removing the released amplification reaction products from the surface.
27. The process according to Claim 21, characterized in that the nucleic acid amplification reaction takes place in a reaction buffer that does not result in release of the nucleic acids from the surface.
28. The process according to Claim 27, which includes the additional steps of:
(d) releasing the amplification reaction products from the surface; and
(e) removing the released amplification reaction products from the surface.
29. A process for performing chemical reactions on nucleic acids including the following steps:
(a) applying at least one nucleic acid-containing sample to a surface;
(b) immobilizing the nucleic acids on the surface;
(c) releasing the immobilized nucleic acids from the surface;
(d) performing at least one chemical reaction with the nucleic acids; and

(e) removing the nucleic acids from the surface without additional immobilization.

30. A process for analysis of nucleic acids in an isolation device including the following steps:

- (a) providing an isolation device with a membrane located therein;
- (b) applying at least one nucleic acid-containing sample to the isolation device;
- (c) immobilizing the nucleic acids on the membrane;
- (d) passing the liquid components of the sample through the membrane; and
- (e) analyzing at least one property of the nucleic acid on the membrane located in the isolation device.

31. The process according to Claim 30, characterized in that after passing the liquid components through the membrane, at least one chemical reaction is performed with the nucleic acids.

32. The process according to Claim 31, characterized in that the chemical reaction is a radioactive labeling of the nucleic acid.

33. The process according to Claim 30, characterized in that the analyzed property is the binding capacity of the nucleic acids for molecules.

34. The process according to Claim 33, characterized in that the molecules are antibodies.

35. The process according to Claim 33, characterized in that the molecules are nucleic acid binding proteins.

36. The process according to Claim 33, characterized in that the molecules are dye molecules.

37. The process according to any one of Claims 9, 14, 21, 29 or 30, characterized in that the sample is introduced onto the top of the membrane or surface.

38. A process according to any one of Claims 1, 9, 19, 28, 29, or 30, characterized in that the immobilized nucleic acids are subjected to a washing step which takes place with at least one washing buffer after the immobilization and before any release steps.

39. The process according to Claim 38, characterized in that the washing step consists of the following steps for each washing buffer:

- applying a predetermined quantity of washing buffer on the surface; and
- passing the washing buffer through the surface.

40. The process according to any one of Claims 1, 9, 19, 28, or 29, characterized in that an aqueous salt or buffer solution is used to release the nucleic acids.

41. The process according to any one of Claims 1, 9, 19, 28, or 29, characterized in that water is used to release the nucleic acids.

42. The process according to one of Claims 1, 9, 14, 21, 29, or 30, characterized in that the introduction and immobilization of the nucleic acids includes the following steps:
(a)(1) mixing at least one nucleic acid-containing sample with an immobilization buffer;
(a)(2) applying said at least one nucleic acid-containing sample with the immobilization buffer to the surface or membrane; and
(a)(3) passing the liquid components through the surface in essentially the same direction they were added.

43. The process according to any one of Claims 1, 9, 19, 28, 29 or 30, characterized in that at least one of the steps is carried out by an automatic device, in a fully automatic manner.

44. The process according to Claim 43, characterized in that all steps of the process are performed by an automatic apparatus in a controlled sequence.

45. The process according to Claim 43, characterized in that a majority of nucleic acid isolations or reactions take place simultaneously.

46. The process according to any one of Claims 1, 9, 19, 28, 29, or 30, characterized in that aqueous salt solutions of metal and/or ammonium cations with mineral acids are used to immobilize the nucleic acids.

47. The process according to Claim 46, wherein the aqueous salt solutions are of alkaline halides, alkaline-earth halides, alkaline sulfates, alkaline-earth sulfates, alkaline phosphates, alkaline-earth phosphates, or mixtures thereof.

48. The process according to Claim 47, characterized in that sodium halides, lithium halides and/or potassium halides and/or magnesium sulfate are used to immobilize the nucleic acids.

49. The process according to any one of Claims 1, 9, 19, 28, 29, or 30, characterized in that aqueous solutions of salts of mono or polybasic or polyfunctional organic acids with alkaline or alkaline-earth metals are used to immobilize the nucleic acids.

50. The process according to Claim 49, characterized in that aqueous solutions of sodium, potassium or magnesium salts with organic dicarboxylic acids are used to immobilize the nucleic acids.

51. The process according to Claim 50, characterized in that the organic dicarboxylic acid is oxalic acid, malonic acid and/or succinic acid.

52. The process according to Claim 49, characterized in that aqueous solutions of sodium or potassium salts with a hydroxy or polyhydroxycarboxylic acid are used to immobilize the nucleic acids.

53. The process according to Claim 52, characterized in that the polyhydroxycarboxylic acid is citric acid.

54. The process according to any one of Claims 1, 9, 19, 28, 29, or 30, characterized in that hydroxy-functional compounds of aliphatic or acyclic saturated or unsaturated hydrocarbons are used for the immobilization of the nucleic acids.

55. The process according to Claim 54, wherein said hydroxy-functional compounds are selected from the C₁-C₅ alkanols.

56. The process according to Claim 55, wherein said alkanols are selected from methanol, ethanol, n-propanol, tert.-butanol, pentanols, and mixtures thereof.

57. The process according to Claim 54, wherein said hydroxy-functional compound is an aldite.

58. The process according to any one of Claims 1, 9, 19, 28, 29, or 30, characterized in that a phenol or polyphenol is used for the immobilization of the nucleic acids.

59. The process according to any one of Claims 1, 9, 19, 28, 29, or 30, wherein at least one chaotropic agent is used for the immobilization of the nucleic acids.

60. The process according to Claim 59, characterized in that the chaotropic agent is a salt selected from the group of trichloroacetates, thiocyanates, perchlorates, iodides, guanidinium hydrochloride, guanidinium isothiocyanate, and urea.

61. The process according to Claim 59, characterized in that 0.01 molar to 10 molar aqueous solutions of at least one chaotropic agent by itself, or in combination with other salts, is used to immobilize the nucleic acids.

62. The process according to Claim 61, characterized in that 0.1 molar to 7 molar aqueous solutions of at least one chaotropic agent by itself, or in combination with other salts, is used to immobilize the nucleic acids.

63. The process according to Claim 62, characterized in that 0.2 molar to 5 molar aqueous solutions of at least one chaotropic agent by itself, or in combination with other salts, is used to immobilize the nucleic acids.

64. The process according to any one of the Claims 61 to 63, wherein the chaotropic agent is selected from an aqueous solution of one or more of sodium perchlorate, guanidinium hydrochloride, guanidinium isothiocyanate, sodium iodide and potassium iodide.

65. The process according to Claim 38, wherein washing steps are carried out using salt or buffer solutions selected from aqueous salt solutions of metal and/or ammonium cations with mineral acids, including alkaline halides, alkaline-earth halides, alkaline sulfates, alkaline-earth sulfates, alkaline phosphates, alkaline-earth phosphates, or mixtures thereof; aqueous solutions of salts of mono or polybasic or polyfunctional organic acids with alkaline or alkaline-earth metals, including sodium, potassium or magnesium salts of organic dicarboxylic acids including oxalic acid, malonic acid and succinic acid; aqueous solutions of sodium or potassium salts of a hydroxy or polyhydroxycarboxylic acid including citric acid; hydroxy-functional compounds of aliphatic or acyclic saturated or unsaturated hydrocarbons including C₁-C₅ alkanols and alditols; phenols or polyphenols; one or more chaotropic agents including salts selected from the group of trichloroacetates, thiocyanates, perchlorates, iodides, guanidinium hydrochloride, guanidinium isothiocyanate, and urea.

66. The process according to any one of Claims 9, 14, 19, 21, 28, or 29, characterized in that the surface is a membrane.

67. The process according to Claim 66, characterized in that the membrane is a hydrophobic membrane.
68. The process according to Claim 67, characterized in that the hydrophobic membrane consists of a polymer with polar groups.
69. The process according to Claim 67 or 68, characterized in that the membrane is a hydrophilic membrane with a hydrophobic surface.
70. The process according to Claim 67 or 68, characterized in that the membrane is made of nylon, a polysulfone, polyethersulfone, polycarbonate, polypropylene, polyacrylate, acrylic copolymer, polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate, poly-tetrafluoro-ethylene, polyvinylidene fluoride, polyethylene-tetrafluoro-ethylene-copolymerisate, a polyethylene-chlorotrifluoro-ethylene-copolymerisate, cellulose acetate, nitrocellulose, polybenzimidazole, polyimide, polyacrylnitrile, polyacrylnitrile-copolymer, cellulose-mix ester, cellulose nitrate, or polyphenylene sulfide.
71. The process according to Claim 70, characterized in that the membrane consists of hydrophobic nylon.
72. The process according to Claim 71, characterized in that the membrane is coated with a hydrophobizing coating agent selected from the group of paraffins, waxes, metal soaps, optionally containing additives selected from the group of aluminum or zirconium salts, quaternary organic compounds, ureic derivates, lipid modified resins, silicones, zinc organic compounds and glutaric dialdehyde.
73. The process according to Claim 1, wherein the membrane is a hydrophilic membrane or a membrane made hydrophilic by pre-treatment.
74. The process according to Claim 73 characterized in that the membrane consists of hydrophilisized nylon, polyethersulfone, polycarbonate, polyacrylate, acrylic copolymer,

polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate, poly-tetrafluoro-ethylene, polyvinylidene fluoride, polyethylene-tetrafluoro-ethylene-copolymerisate, a polyethylene-chlorotrifluoro-ethylene-copolymerisate, cellulose acetate, polypropylene, nitrocellulose, polybenzimidazole, polyimide, polyacrylnitrile, polyacrylnitrile-copolymer, cellulose-mix ester, polyester, polysulfone, cellulose nitrate, or polyphenylene sulfide.

75. The process according to any one of Claims 9, 14, 19, 21, 28, or 29, characterized in that the membrane has a pore diameter of 0.001 to 50 micrometer.

76. The process according to any one of Claims 9, 14, 19, 21, 28, or 29, characterized in that the surface is a hydrophobic fleece.

77. A process for isolating nucleic acids including the following steps:
(a) providing an isolation device with at least one membrane located therein;
(b) applying a nucleic acid-containing sample to the isolation device;
(c) precipitating the nucleic acids contained in the sample with an alcohol, so that the nucleic acids are bound to the at least one membrane,
characterized in that the pore size of said at least one membrane is equal or larger than 0.2 micrometer.

78. The process according to Claim 77, characterized in that the alcohol is added to the nucleic acid-containing sample prior to adding the sample to the isolation device.

79. The process according to Claim 77, characterized in that the alcohol is added to the nucleic acid-containing sample after adding the sample to the isolation device.

80. The process according to Claim 77, characterized in that the surface of the membrane is selected so that all the nucleic acids contained in the solution can be bound to the membrane.

81. The process according to Claim 77, wherein said membrane has a pore size equal to or greater than 0.2 micrometer.
82. The process according to Claim 81, wherein said nucleic acids precipitated are DNA and/or RNA.
83. The process according to Claim 77, wherein the alcohol used is a C₁-C₅ alkanol with.
84. The process according to Claim 77, wherein the alcohol is isopropanol, and the volume ratio of the nucleic acids-containing sample to isopropanol is 2:1 to 1:1.
85. The process according to Claim 77, wherein the membrane is a hydrophobic membrane.
86. The process according to Claim 85, wherein the hydrophobic membrane consists of a polymer with polar groups.
87. The process according to Claim 85, wherein the membrane is a hydrophilic membrane with a hydrophobic surface.
88. The process according to Claim 85, characterized in that the membrane consists of nylon, polyethersulfone, polypropylene, polycarbonate, polyacrylate, acrylic copolymer, polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate, poly-tetrafluoro-ethylene, polyvinylidene fluoride, polyethylene-tetrafluoro-ethylene-copolymerisate, a polyethylene-chlorotrifluoro-ethylene-copolymerisate or, polyphenylene sulfide.
89. The process according to Claim 88, characterized in that the membrane consists of a hydrophobic nylon.
90. The process according to Claim 87, characterized in that the membrane is coated with a waterproofing agent selected from the group of paraffins, waxes, metal soaps, optionally containing additives selected from aluminum or zirconium salts, quaternary organic

compounds, ureic derivatives, lipid modified melamine resins, silicones, zinc organic compounds and/or glutaric dialdehyde.

91. The process according to Claim 77, wherein the membrane is a hydrophilic membrane or a hydrophilized membrane.
92. The process according to Claim 91, wherein the membrane consists of hydrophilized nylon, polyethersulfone, polycarbonate, polyacrylate, acrylic copolymer, polyurethane, polyamide, polyvinylchloride, polyfluorocarbonate, poly-tetrafluoro-ethylene, polyvinylidene fluoride, polyethylene-tetrafluoro-ethylene-copolymerisate, a polyethylene-chlorotrifluoroethylene-copolymerisate, cellulose acetate, cellulose nitrate, or polyphenylene sulfide.
93. The process according to Claim 92, wherein the membrane consists of cellulose acetate or cellulose nitrate.
94. The process according to Claim 91, wherein the membrane has a pore size of more than 0.45 μm .
95. The process according to Claim 91, wherein the membrane has a pore size of more than 0.6 μm .
96. An apparatus capable of performing at least one of the steps of the process according to any one of Claims 1, 9, 14, 21, 29, 30 or 77 automatically.
97. The apparatus according to Claim 96, which is equipped with at least one suction mechanism and which performs or is able to perform the addition of buffers and solutions onto the surface.

98. An isolation device adapted to the isolation of nucleic acids comprising:
at least one cylindrical upper part with an upper opening, a bottom opening, and a membrane located at the bottom opening and fills the entire diameter of the upper part;
a bottom part containing an absorbent material; and
a mechanism for connecting the upper and bottom parts, such that, after the connection is made, the membrane is in contact with the absorbent material and, in case the connection is not made, the membrane is not in contact with the absorbent material.
99. The isolation device according to Claim 98, characterized in that the bottom part is a cylinder having the same diameter as the upper part.
100. The isolation device according to Claim 98, characterized in that the mechanism for connecting the upper and bottom parts also permits the spatial separation of the upper and bottom parts.
101. The isolation device according to Claim 100, wherein the connection mechanism is a bayonet socket.
102. The isolation device according to Claim 100, wherein the connection mechanism is a threaded socket.
103. The isolation device according to Claim 98, characterized in that the mechanism for connecting the upper and bottom parts includes a sliding mechanism, which can be slid between the absorbent material and the membrane, to separate the upper and bottom parts.
104. The isolation device according to Claim 98, characterized in that the connection mechanism has a predetermined breaking point between the upper and bottom part.

105. The isolation device according Claim 98, characterized in that the upper part is a tube, which can be placed in a reaction device container.
106. The isolation device according to Claim 98, characterized in that the upper and bottom parts form a tube, which can be placed in a reaction device container.
107. The isolation device according to Claim 98, wherein said bottom part is configured to connect with a plurality of upper parts.
108. The isolation device according to Claim 98, wherein the absorbent material is a sponge.
109. The isolation device according to Claim 98, wherein the absorbent material contains a granulate.
110. An isolation device adapted for the isolation of nucleic acids comprising:
at least one upper part having an upper opening, a bottom opening, and a membrane which is located at the bottom opening and which fills the entire diameter of the isolation device;
a bottom part having an absorbent material;
and a collar surrounding the upper part at least in the area of the membrane to accommodate a coolant.
111. The isolation device according to Claim 110, wherein said collar has two compartments, which are separated from one another by a frangible separation wall; and wherein each of the compartments contains a solution, whereby a coolant is produced when both solutions are mixed after breaking the separation wall.
112. A method for isolating nucleic acids comprising contacting a sample containing nucleic acids with a material selected from the group of cellulose acetate; non-carboxylized, hydrophobic polyvinylidene fluoride; and massive, hydrophobic polytetrafluoroethylene.

113. The method of Claim 112, wherein said material is used in the form of a membrane.

114. The method of Claim 112, wherein said material is used in the form of a granulate.

115. The method of Claim 112, wherein the material is used in the form of a fiber.

116. The method of Claim 115, wherein the fibers are organized as a fleece.

117. A kit for the isolation of nucleic acids comprising:

- an immobilization buffer;
- an elution buffer; and
- at least one isolation device according to one of Claims 98 to 111.

118. The kit of Claim 117, characterized in that it also contains a washing buffer.

119. The kit of Claim 117 or 118, characterized in that it also contains a lysis buffer.

120. The kit according to Claim 117 or 118, wherein said at least one isolation device is configured and instructions are provided for performing a process according to any one of Claims 1, 9, 14, 19, 21, 28, 29, 30, or 77.

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